

THE SOLUBILITY OF TYROSINE IN ACID AND IN ALKALI.

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I.

INTRODUCTION.

Many amphoteric electrolytes form soluble salts with acid or alkali, while the undissociated or isoelectric substances themselves are often only slightly soluble in water. It should, therefore, be possible to calculate the solubility of such a substance from its ionization constants as an acid and as a base and from the hydrogen ion concentration of the saturated solution. The theory underlying this dependence of the solubility on the hydrogen ion concentration has been outlined by Michaelis,¹ who stated, however, that the only experimental investigations of the question have consisted in determinations of the pH at which the solubility was a minimum. A few measurements were made by Beveridge² of the solubility of anthranilic acid in hydrochloric acid, but the work was hardly extended far enough to determine whether the increased solubility could be theoretically explained. Cohn and Hendry³ studied the effect of hydrogen ion concentration on the solubility of casein in very dilute solutions of sodium hydroxide, but this case was complicated by the fact that the constitution, molecular weight, and basicity of the ampholyte were unknown. Accordingly it seemed advisable to measure the solubility in acid and in alkali of a comparatively simple ampholyte of known constitution, with the object of testing the theoretical relation between the solubility and hydrogen

¹ Michaelis, L., *Die Wasserstoffionenkonzentration*, Berlin, 2nd edition, 1922, 73, 58.

² Beveridge, H. H., *Proc. Roy. Soc. Edinburgh*, 1908-09, xxix, 648.

³ Cohn, E. J., and Hendry, J. L., *J. Gen. Physiol.*, 1922-23, v, 521.

ion concentration. The substance used in this work was *L*-tyrosine, or para-hydroxyphenyl- α -amino-propionic acid, $\text{HO}\cdot\text{C}_6\text{H}_4\cdot\text{CH}_2\cdot\text{CHNH}_2\cdot\text{COOH}$.

II.

EXPERIMENTAL PROCEDURE.

The tryosine was prepared from commercial casein hydrolyzed by boiling with hydrochloric acid, following the procedure of Abderhalden⁴ (who, however, used silk as the raw material). The product was decolorized with animal charcoal, recrystallized three times from hot water, and dried with alcohol and ether. Its nitrogen content, determined by the Kjeldahl method, was found to be 7.73 per cent; calculated, 7.733.

Saturated solutions of tyrosine in HCl or NaOH of various concentrations were obtained by placing 100 cc. of the solvent in contact with an excess of solid tyrosine in 150 cc. Pyrex flasks closed tightly by rubber stoppers, and rotating the flasks, end over end, for 1 or 2 days in a water bath at $25^\circ\text{C.} \pm 0.05^\circ$. Preliminary measurements of the solubility gave identical values after 1 and after 2 days in the thermostat. In the case of pure water it was found that equilibrium was reached more slowly; concordant values were obtained only after 3 days agitation of tyrosine with supersaturated and undersaturated solutions. The solutions were filtered with suction through hardened filter paper fastened by a rubber band over the end of a glass tube about 6 mm. in diameter, which was connected by rubber tubing to a pipette. This made it possible to conduct the filtration without removing the flasks from the thermostat.

The concentration of tyrosine in the saturated solutions was obtained by Kjeldahl determinations of nitrogen, using 100 cc. Kjeldahl flasks with 2 cc. of concentrated H_2SO_4 , 0.5 cc. of 5 per cent CuSO_4 , 1 gm. of K_2SO_4 , and a few pieces of broken alundum to prevent bumping. For the lower concentrations of tyrosine 25 cc. samples were used; for the higher concentrations, suitable aliquot parts. After the addition of 50 cc. of water and 6 cc. of 1:1 sodium

⁴ Abderhalden, E., *Z. physiol. Chem.*, 1912, lxxvii, 75.

hydroxide solution, the ammonia was distilled into 25 cc. of N/50 HCl, and determined by titration with N/50 NaOH, using methyl red as indicator. The blanks obtained with the reagents varied from 0.2 to 0.3 cc. N/50. Duplicate analyses generally agreed to within 0.1 cc. N/50. Each solubility determination represents the mean of two analyses; the figure for tyrosine and water is the mean of those obtained from eight separate solutions.

The hydrogen ion concentrations were determined by electromotive force measurements with Clark electrodes in an air thermostat at 25°C. They are based on the value 1.035 for the pH of 0.1000 M HCl, and on the assumption that contact potential differences were eliminated by the use of saturated KCl. The concentrations of hydroxyl ions in the alkaline solutions were calculated from the pH measurements, using the value $k_w = 1 \times 10^{-14}$ for the ion product of water at 25°.

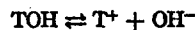
The concentrations of HCl and NaOH given are those of the original solutions with which the tyrosine was shaken. It was found by direct titration, using methyl red, that the solution of the tyrosine did not appreciably change these values.

The results are given in Tables I and II.

III.

DISCUSSION OF RESULTS.

The results obtained with hydrochloric acid are plotted in Fig. 1. It is evident that the solubility of tyrosine is a linear function of the initial concentration of hydrochloric acid, and also of the final concentration of hydrogen ion. That this is predicted by the theory may be shown as follows. Representing tyrosine by TOH , in acid solutions it will ionize as a base:



Applying the law of mass action,

$$\frac{[\text{T}^+][\text{OH}^-]}{[\text{TOH}]} = k_b$$

Since $[H^+][OH^-] = k_w$, the ion product of water, letting $K = \frac{k_b}{k_w}$, $s_0 = [TOH]$, $h = [H^+]$, and $t = [T^+]$, it follows that

$$K = \frac{t}{s_0 h} \quad (1)$$

TABLE I.

Solubility of Tyrosine in Hydrochloric Acid.

Concentration of HCl, mols per liter $\times 10^3$.	Concentration of tyrosine, mols per liter $\times 10^3$.	pH	Concentration of hydrogen ion, mols per liter $\times 10^3$.
0	2.62	5.1 to 5.5	0.01
1.00	3.09	3.19	0.65
2.00	3.25	2.857	1.39
4.99	4.10	2.457	3.49
9.99	5.39	2.160	6.92
20.00	8.43	1.861	13.8
30.00	10.8	1.675	21.1
40.00	13.8	1.560	27.6
50.00	16.5	1.450	35.5

TABLE II.

Solubility of Tyrosine in Sodium Hydroxide.

Concentration of NaOH, mols per liter $\times 10^3$.	Concentration of tyrosine, mols per liter $\times 10^3$.	pH	Concentration of hydroxyl ion, mols per liter $\times 10^3$.
0	2.62	5.1 to 5.5	0.000
0.98	3.54	8.342	0.220
1.95	4.30	8.865	0.731
4.88	7.06	9.249	1.775
9.76	10.7	9.484	3.05
19.5	17.5	9.726	5.32
29.9	24.7	9.841	6.94
39.8	30.4	9.881	7.60
49.8	35.8	9.953	8.98

The total solubility of tyrosine, s , is equal to the concentration of the undissociated molecule plus that of the tyrosine chloride formed, or

$$s = s_0 + \frac{t}{d_s} \quad (2)$$

if d_s = the degree of dissociation of the salt formed. The total concentration of hydrochloric acid, a , is equal to that of the free hydrochloric acid remaining plus that of the tyrosine chloride formed, or

$$a = \frac{h}{d_a} + \frac{t}{d_s} \quad (3)$$

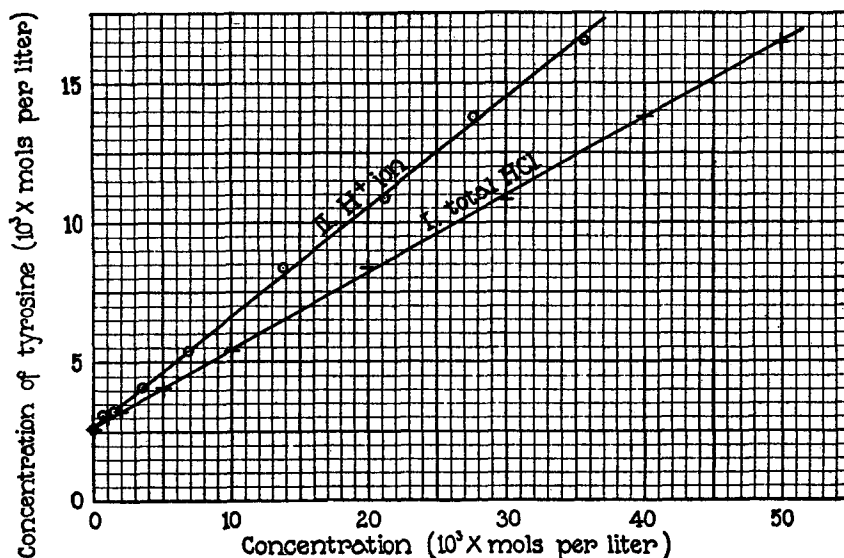


FIG. 1. Effect of the concentration of total HCl and of H^+ ion on the solubility of tyrosine. The curves are linear, as the theory requires for a mono-acid base.

where d_a is the degree of dissociation of the hydrochloric acid. By combining these three equations the following expression is obtained for s in terms of a .

$$s = s_0 + \frac{K s_0}{\frac{d_s}{d_a} + K s_0} a$$

Assuming that $d_s = d_a$ (which should be approximately true in accordance with the isoionic principle and the rule of equal ionization for strong electrolytes of the same type⁵), the relation becomes

⁵ Washburn, E. W., Principles of physical chemistry, New York and London, 1st edition, 1915, 317, 295, 220.

$$s = s_0 + \frac{K s_0}{1 + K s_0} a \quad (4)$$

This equation indicates the linear relationship between s and a , in Curve I of Fig 1. Evidently s_0 is, within the experimental error, equal to the solubility of tyrosine in pure water, and the slope of the line is $\frac{K s_0}{1 + K s_0}$.

By combining equations (1) and (2) a relation between s and h can be obtained, which is

$$s = s_0 + \frac{K s_0}{d_s} h$$

This is a linear equation only if d_s is constant. If the approximate assumption is made that $d_s = 1$, then

$$s = s_0 + K s_0 h \quad (5)$$

Equation (5) thus represents the line which is Curve II of Fig. 1, s_0 being the solubility of tyrosine in water, and $K s_0$ being the slope of the line.

It is also possible to show that a linear relation exists between h and a . By combining equations (1) and (3) it follows that

$$a = h \left(\frac{1}{d_a} + \frac{K s_0}{d_s} \right)$$

If it is assumed that $d_a = d_s = 1$, then

$$a = h (1 + K s_0) \quad (6)$$

Equations (4), (5), and (6) can be tested by using the experimental values of s_0 , s , a , and h to calculate K , and hence k_b , which is the product of K and k_w . The results of this calculation are given in Table III, using 1.0×10^{-14} as the value of k_w at 25° . The best value for k_b , 1.57×10^{-12} , is probably more reliable than the value 2.6×10^{-12} , which was found by Kanitz.⁶ The older value was obtained by the conductivity method, which involves a doubtful estimation of the conductivity of unhydrolyzed tyrosine chloride. Attempts to obtain this quantity by experiment in this laboratory have been fruitless.

⁶ Kanitz, A., *Arch. ges. Physiol.*, 1907, cxviii, 539.

TABLE III.
Basic Ionization Constant of Tyrosine.

Values for $k_b \times 10^{12}$ as calculated from.			s (observed) $\times 10^3$.	s (calculated) $\times 10^3$.
a and s .	h and s .	h and a .		
(3.4)	(2.8)	(2.1)	2.62	2.62
1.8	1.7	1.7	3.09	2.89
1.6	1.6	1.6	3.25	3.19
1.5	1.5	1.7	4.10	4.06
1.6	1.5	1.7	5.39	5.47
1.6	1.6	1.7	8.43	8.29
1.4	1.5	1.6	10.8	11.3
1.5	1.6	1.7	13.8	14.0
1.5	1.5	1.6	16.5	17.2
Mean. . . . 1.56	1.57	1.66	$k_b = 1.57 \times 10^{-12}$	

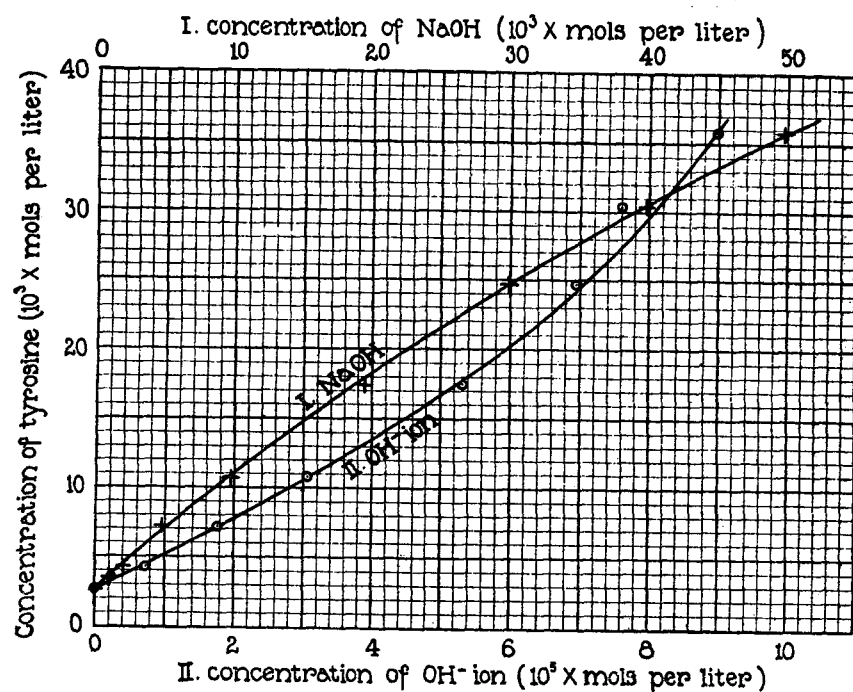


FIG. 2. Effect of the concentration of total NaOH and of OH^- ion on the solubility of tyrosine. The curves are not linear because tyrosine is a dibasic acid.

The constancy of the values for k_b indicates the extent to which the experimental data agree with the theory and the assumptions made. The agreement of the observed values of s with those calculated from equation (5) indicates more directly, perhaps, that the data are satisfactorily explained by the theory.

The results obtained with tyrosine and sodium hydroxide are plotted in Fig. 2. Here the curves are not linear; and the explanation for this is to be found in the fact that tyrosine acts as a dibasic acid, the phenol group as well as the carboxyl group losing hydrogen ions.

Representing the total concentration of sodium hydroxide by b , its degree of ionization by d_b , the ionization of monosodium tyrosinate by d_1 , the concentration of its negative ion by t_1 , the ionization of disodium tyrosinate by d_2 , and the concentration of its negative ion by t_2 , the following equations result from the application of the law of mass action to the ionization of tyrosine as a dibasic acid.

$$\frac{t_1}{s_0} \frac{h}{h} = k_{a1} \quad (7)$$

$$\frac{t_2}{t_1} \frac{h}{h} = k_{a2} \quad (8)$$

$$\frac{t_2}{s_0} \frac{h^2}{h^2} = k_{a1} k_{a2} \quad (9)$$

It follows also that

$$b = \frac{k_w}{h d_b} + \frac{t_1}{d_1} = \frac{2 t_2}{d_2} \quad (10)$$

$$s = s_0 + \frac{t_1}{d_1} + \frac{t_2}{d_2} \quad (11)$$

Substituting the values of t_1 and t_2 from equations (7) and (9), and making the somewhat doubtful assumption that $d_b = d_1 = d_2 = 1$, we have

$$b = \frac{k_w}{h} + \frac{k_{a1} s_0}{h} + \frac{2 k_{a1} k_{a2} s_0}{h^2} \quad (12)$$

and

$$s = s_0 + \frac{k_{a1} s_0}{h} + \frac{k_{a1} k_{a2} s_0}{h^2} \quad (13)$$

By combining equations (12) and (13) it is possible to calculate approximate values for k_1 and k_2 . Thus it would follow that

$$b - s = \frac{k_w}{h} + \frac{k_{a1} k_{a2} s_0}{h^2} - s_0$$

and

$$b - 2s = \frac{k_w}{h} - 2s_0 - \frac{k_{a1} s_0}{h}$$

from which

$$k_{a1} = \left(\frac{k_w}{h} + 2s - 2s_0 - b \right) \frac{h}{s_0} \quad (14)$$

and

$$k_{a1} k_{a2} = \left(b + s_0 - s - \frac{k_w}{h} \right) \frac{h^2}{s_0} \quad (15)$$

It is to be noted that equations (7) to (11) are exact, while equations (12) to (15) are only approximate, since they contain the assumption of complete ionization. However, the values of k_{a1} and k_{a2} calculated from equations (14) and (15) exhibit a certain degree of constancy, as is shown in Table IV. The table contains also a comparison of the observed values of s with those calculated from equation (13), using the mean values for k_{a1} and k_{a2} and the observed values for s_0 and h .

The complete expression for the solubility of tyrosine at any hydrogen ion concentration is

$$s = s_0 \left(1 + \frac{k_b h}{k_w} + \frac{k_{a1}}{h} + \frac{k_{a1} k_{a2}}{h^2} \right) \quad (16)$$

If the values already given for the constants are substituted, this becomes

$$s = 0.00262 \left(1 + 157 h + \frac{7.8 \times 10^{-10}}{h} + \frac{6.6 \times 10^{-20}}{h^2} \right) \quad (17)$$

The curve in Fig. 3 represents values of s calculated from this equation, plotted as a function of the pH. The circles represent the values of s experimentally determined.

TABLE IV.

Acidic Ionization Constants of Tyrosine.

$k_{a1} \times 10^{10}$	$k_{a1}k_{a2} \times 10^{20}$	s (observed) $\times 10^3$	s (calculated) $\times 10^3$
(14.9)	(47)	3.54	3.08
7.4	(18)	4.30	4.20
8.7	5.1	7.06	6.79
8.1	6.8	10.7	10.5
7.4	6.1	17.5	18.4
7.9	6.1	24.7	25.2
8.0	7.9	30.4	28.2
7.1	7.8	35.8	34.9
Mean....7.8	Mean....6.6	$k_{a1} = 7.8 \times 10^{-10}$ $k_{a2} = 8.5 \times 10^{-11}$	

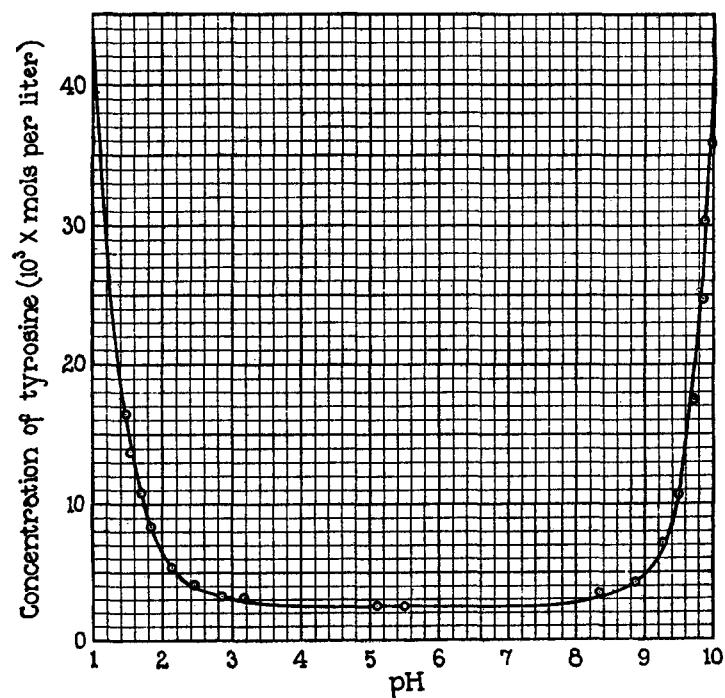


FIG. 3. Effect of pH on solubility of tyrosine. The points represent observed values; the curve represents values calculated from the theory.

In equation (16) the expression in parentheses is the reciprocal of the quantity designated by Michaelis⁷ as ρ , the unionized fraction of the ampholyte, which in the notation used here is $\frac{s_0}{s}$. By differentiating his expression with respect to h and placing the derivative equal to zero, an equation can be derived for the hydrogen ion concentration at the isoelectric point, as was done by Michaelis for the case of an ampholyte with only one acidic group. Substituting the values of the constants, this calculation gives for the isoelectric point

$$h^3 = 5 \times 10^{-12} h + 8.4 \times 10^{-22} \quad (18)$$

From the shape of the curve in Fig. 3, the isoelectric point would appear to lie between pH 5 and 6. If this is the case the last term in equation (18) is negligible, and the value for h becomes 2.23×10^{-6} , corresponding to pH 5.65. The same value is obtained by the equation of Michaelis, which considers from the start only one acid and one basic ionization constant. In such a case as this, however, as Michaelis has pointed out, the exact location of the isoelectric point has little significance, since the ampholyte is isoelectric or almost completely undissociated over a broad zone of pH values.

SUMMARY.

Measurements have been made of the solubility at 25°C. of tyrosine in hydrochloric acid and in sodium hydroxide solutions varying from 0.001 to 0.05 M, and also in distilled water. The pH of the saturated solutions was measured with the hydrogen electrode. The following values for the ionization constants of tyrosine have been obtained from the measurements: $k_b = 1.57 \times 10^{-12}$, $k_{a1} = 7.8 \times 10^{-10}$, $k_{a2} = 8.5 \times 10^{-11}$. The changes in solubility with pH can be satisfactorily explained by the use of these ionization constants.

⁷ Michaelis¹, p. 54.